English Translation of Annex to International Preliminary Examination Report

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Use of R-arylpropionic acids for the production of medicaments for the treatment of diseases in humans and animals which can be therapeutically influenced by the inhibition of the activation of NF-κB.

The subject of the present invention is the use of R-arylpropionic acids for the production of medicaments for the treatment of diseases in humans and animals which can be therapeutically influenced by the inhibition of the activation of NF-κB.

Arylpropionic acids and their derivatives have long since been used as nonsteroidal anti-inflammatory and analgesically effective medicaments. Known representatives of the active material group are ibuprofen, flurbiprofen, ketoprofen, napoxen, tiaprofenic acid and fenoprofen /propionic acid derivatives: Goodman and Gilman's, The pharmacological basis of therapeutics, Chapter 27, p. 637 (ninth edition, 1996/7.

On the basis of the molecular structure with an asymmetrical C-atom, arylpropionic acids and their derivatives are chiral, thus occur as R- and S-enantiomeric forms. In the case of the chemical synthesis, these active materials normally occur as racemate. Apart from S-naproxen /Williams: Enantiomers in arthritic disorders; Pharmac. Ther., Vol. 26, pp. 273-295 (1990); Evans: Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal anti-inflammatory drugs, Eur. J. Pharmacol., 42, 237-256 (1992-7 and recently dexibuprofen

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/Symposium: Update on S(+)-ibuprofen; Going /Kitzbühl, 2 to 4 February, 1996/ and dexketoprofen /Scrip No. 1831, June 22nd 1993, p. 7, Scrip No. 2144, July 9th 1996, p. 16/, these active materials have hitherto been used as racemates.

The therapeutically desired inflammation-inhibiting and pain ameliorating action of arylpropionic acuds and their derivatives is essentially ascribed to the inhibition of the prostaglandin biosynthesis /Vane and Botting: Overview - mechanism of action of anti-inflammatory drugs. In: Improved non-steroidal anti-inflammatory drugs - COX-2 enzyme inhibitors, p. 1 - 27, Lancester - Kluwer Academic Publishers (1996)/. This takes place via the inhibition of the enzymes cyclooxygenases 1 and 2 (COX-1 and COX-2 or PGHS-1 and PGHS-2) participating in the formation of prostaglandins. Due to the reduced formation of prostaglandins, the inflammation symptoms, such as pain, swelling, reddening, oedema formation, heating and function limitation, the inflammation symptoms standing in conjunction with these inflammation mediators are weakened. The inhibition of the prostaglandin biosynthesis is taken as general characteristic of the mechanism of the anti-inflammatory and of the analgesic action. The therapeutically desired inhibition of the prostaglandin production in the diseased object tissue leads in other organ systems, which indicate the presence of certain prostaglandin concentrations, to undesired

medicament actions. Especially affected by the undesired actions are the stomach-intestine tract, the kidneys, the lungs and the blood platelets.

It is known that, with reference to the prostaglandin synthesis inhibition, substantial differences exist between the enantiomeric forms of the arylpropionic acids /Williams (v. supra); Evans (v. supra); Brooks and Day: New nonsteroidal anti-inflammatory drugs, Birkhauser Verlag, Basel, p. 119-126 (1985)7. Whereas all S-enantiomers of these substances show an outstanding prostaglandin synthesis inhibition, in the case of the R-enantiomers this is not found in the therapeutically relevant concentration range. Consequently, in therapeutic concentrations, to the R-arylpropionic acids and their derivatives are ascribed neither the desired nor the undesired medicinal actions which stand in conjunction with the inhibition of the prostaglandin production. Independently of the absence of these action mechanism-specific undesired actions, the R-enantiomers of this active material class display substance-specific undesired actions.

Because of the hitherto therapeutic and economic importance of the arylpropionic acids used as racemate, it is sought to establish the reasonableness of the use of racemic active materials. In the case of ibuprofen, the use of the racemate is essentially based on the fact that, in the human or animal organism, a more or less marked inversion of R-isoprofen to S-ibuprofen takes

place /Caldwell et al., The metabolic chiral inversion and dispositional enantioselectivity of the 2-aryl-propionic acids and their biological consequences; Biochemical Pharmacology, Vol. 37, No. 1, pp. 105-114 (1988)/ so that also a part of the R-form, after inversion to the S-form, can be effective as prostaglandin synthesis inhibitor. Furthermore, for R-ibuprofen, an inhibition of the polymorphonuclear leukocytes in vitro is described which could prove to be advantageous in the case of inflammatory diseases /Villanueva et al., Equipotent inhibition by R(-)-, S(+)- and racemic ibuprofen of human polymorphonuclear cell function in vitro; Br. J. Pharmac., 35, 235-242 (1993)/. However, the therapeutic relevance of this mechanism in the case of use of racemic iboprofen could not be shown. For R-flurbiprofen, the inversion can be neglected.

The fact that the therapeutic action of the aryl-propionic acids is essentially ascribed to the prostaglandin synthesis inhibition has led to the recognition that the use of the pure S-enantiomers, possibly of the racemic compounds but not of the pure R-enantiomers is meaningful. First with the surprising discovery that R-flurbiprofen displays an antinociceptive effect which does not stand in connection with the inhibition of the peripheral prostaglandin biosynthesis was the development of medicaments based on R-flurbiprofen /DE 40 28 906 C2; EP 0 607 128 B1; USA 5,206,029 and

5,200,1987 as analgesic without inflammation-inhibiting active component initiated. Later, a pain-ameliorating action was also described for R-ketoprofen /DE 43 19 438 C1; WO 93/17667.

Recent publications confirm the antinociceptive effect of R-flurbiprofen /Geisslinger, Schaible: New insights into the site and mode of antinociceptive action of flubiprofen enantiomers, J. Clin. Pharmacol., 36, 513-520 (1996), Buritova, Besson, Peripheral and/or central effects of racemic, S(+)- and R(-)-flurbiprofen on inflammatory nociceptive processes: a c-Fos protein study in the rat spinal cord; British J. Pharmacology, 125, 87-101 (1998)7. In clinical studies on patients, the pain-ameliorating action of R-flurbiprofen /Fig. 17 and R-ketoprofen /Cooper et al., Analgesic efficacy and safety of R-ketoprofen in postoperative dental pain; J. Clin. Pharmacol., 38, 11S-18S (1998)7 could be demonstrated.

Fig. 1 Placebo-controlled double blind study on 180 women with acute post-episiotomy pain (average value curves)

The hospitalised pateints were randomised in three medication groups each with 50 patients and a placebo group (30 patients). Within 48 hours, each patient received, after otherwise normally proceeding delivery, a single dose of the study medication to be investigated (25 mg R(-)-flurbiprofen or 100 mg (R)-flurbiprofen or

1000 mg paracetamol) or a placebo, administered orally. Shortly before the oral administration of the test preparation or of the placebo and at precisely fixed investigation point of time (15, 30, 45, 60, 120, 180, 240, 300 and 360 minutes), the patients were questioned with regard to their feeling of pain. The effectiveness of the individual preparations were assessed on the basis of a pain feeling scale (0 = none, 1 = mild, 2 = moderate, 3 = strong). The time courses are summarised in the average value curves of the individual patients given in Figure 1.

Animal experimental studies verify that the action of R-flurbiprofen can be explained via the inflammation-inhibiting and antinociceptive action on the central nervous system /Buritova (v. supra); Neugebauer et al., Antinociceptive effects of R(-)- and S(+)— flurbiprofen on rat spinal dorsal horn neurons rendered hyper-excitable by an acute knee joint inflammation; J. Phermacol. Exp. Ther; 275, 618-628 (1995)7. The known peripheral inflammation-inhibiting and antinociceptive action of flurbiprofen could, on the other hand, be found exclusively in the case of the S-enantiomers /Buritova (v. supra) and Neugebauer (v. supra)7. According to the present state of knowledge, there results therefrom the significant concequence that, for the optimal treatment of the peripheral inflammatory diseases, S-arylpropionic acids are to be used as agents of choice. For the reduction of the undesired activities on the stomach-

intestinal tract etc. connectedwith the prostaglandin synthesis inhibition, e.g. S-flurbiprofen should not be taken orally but rather administered locally to the inflammed or painful place. However, because of the central action, R-flurbiprofen should be administered systemically /Buritova (v. supra)/, e.g. orally, intramuscularly or intravenously.

Contrary to this newest knowledge for the practically exclusive central action of R-flurbiprofen, it was now surprisingly found that R-flurbiprofen in certain concentrations is a potent and specific inhibitor of the activation of the nuclear transcription factor NF-κB. NF-κB is a ubiquitous transcription factor which takes up a central role in cells in the case of immune and inflammation reactions, as well as in the expression of cytokines, chemokines, cell adhesion molecules, growth factors, immune receptors, acute phase proteins , diverse enzymes and other transcription factors /Lee, Burckart: Nuclear factor kappa B: Important transcription factor and therapeutic target, J. Clin. Pharm., 38, 981-993 (1998)/.

The NF-κB activation can be inhibited by various active materials at different steps of the activation cascade. Thus, glucocorticoids inhibit NF-κB by direct association or by strengthening of the expression. Cyclosporins and tacrolimus prevent the NF-κB activation by inhibition of the calcineurin action of the phosphatases

which indirectly induce the I-κB decomposition. Deoxyspargualin inhibits NF-κB by blockade of its nucleus displacement. Aspirin and salicylates inhibit present occurances which induce the I-κB phosphorylation. Tepoxalin and antioxidants inhibit the NF-κB activation by changing of the redox state of the cell. Further researches are necessary in order to develop specific inhibitors of the treatment of diseases which are influenced by NF-κB /Lee, Burckart: Nuclear factor kappa B; Important transcription factor and therapeutic target, J. Clin. Pharm., 38, 981-992 (1998)/.

It is known that R-ibuprofen and S-ibuprofen inhibit the activation of the transcriptuon factor NF-κB by phorbol esters (TPA), which is attributed to a regulation of the protein kinase C (PKC) activated by phorbol esters and thereby brought about phosphorylation and inactivation of the I-κB but is not able to influence an NF-κB activation by PGE_2 or lipopolysaccharides (LPS). The usability of ibuprofen is, therefore, limited /N. Scheuren et al., "modulation of transcription factors by nonsteroidal anti-inflammatory drugs", Naunyn-Schmiedeberg's Arch. Pharmacol., Vol. 354, No. 4, suppl. 1, 1996; N. Scheuren et al., "Enantiomers of the nonsteroidal anti-inflammatory drug ibuprofen are potent and specific inhibitors of transcription factor NF-kappa, beta, "Naunyn-Schmiederberg's Arch. Pharmacol., Vol. 357, No. 4 suppl., 1998; N. Schueren et al. "Modulation of transcription factor NF-kappa,beta

by enantiomers of the nonsteroidal drug ibuprofen, Br. J. Pharmacol., Vol. 123, No. 4, 1998; N. Scheuren et al., "Weak inhibitors of cyclooxygenases may exert their antinociceptive effects by modulation of transcription factors, Adv. Exp. Med. Biol., Vol. 433, 1997.

The invention has now set itself the task to find further active materials which inhibit NF-κB activation.

Surprisingly, it has now been found that other non-racemising R-arylpropionic acids can intervene via the specific inhibition of steps within the NF-κB cascade in the disease happenings. Because of the ubiquitous function of the transcription factor NF-κB in the case of the gene regulation, medicaments with N-arylpropionic acids or their derivatives are suitable not only for the known pain amelioration via the antinociceptive action on the central nervous system /DE 40 28 906 C2/ but, in the case of suitable use and dosage, can also be used in the case of all diseases in which an inhibition of the NF-κB can be therapeutically advantageously used. According to the invention, these medicaments can be used not only in the case of pain and rheumatism but also in the case of immune diseases, asthma, shock, inflammatory intestinal diseases (Crohn's disease, colitis ulcerosa), radiation damages, arteriosclerosis, in the treatment of rejection reactions after tissue and organ transplants etc., in each case in appropriate doses and pharmaceutical formulations.

The here reported observation of the inhibition of the NF-κB formation is surprising because, according to the prior art, the pharmacological effects of the arylpropionic acids were ascribed to other mechanisms. This has hitherto led to the use of the racemates or of the S-enantiomers in comparatively small doses in the case of pains and inflammations.

Furthermore, in WO 98/09603 is described the usability of R-NSAID's in neoplastic diseases, especially colon and breast cancer, cystic fibrosis and Alzheimer's disease.

Surprisingly, it has now been found that R-flurbiprofen and other R-arylpropionic acids not metabolising to CoA-thioesters and thus racemising inhibit the NF-κB activation about 100 times more potently than the corresponding S-enantiomers. However, in order to achieve a sufficient action, they must be used in higher dosages than are usual in the case of the known therapeutic use of racemic arylpropionic acids. However, because of the good compatability on the basis of the practically absent action of these R-arylpropionic acid dosages on the peripheral prostaglandin biosynthesis and the racemising to the S-enantiomers not taking place, it is possible, in the case of use of the R-enantiomers, to make the dose so high that the desired inhibiting action on the NF-κB activation is achieved without having to fear the undesired actions

brought about by the S-form. The active materials are, therefore, preferably used substantially free of the S-enantiomers, i.e. with an optical purity of over 90%, especially over 99%, if, as "side effect", the known pain- and inflammation-inhibiting action of the S-enantiomers is also not desired. In contradistinction to R-ibuprofen, in this regard undesired actions because of the absent R $\Longrightarrow$ S inversion in the case of the R-arylpropionic acids not metabolising to the CoA thioesters are not to be expected. Thus, the medicaments according to the invention permit an improved therapeutic breadth to be expected in comparison with the use of the racemic arylpropionic acids or of their S-enantiomers, The investigations carried out on humans verify the good gastrointestinal compatability of R-flurbiprofen and other R-arylpropionic acids /Jerussi et al., Clinical endoscopic evaluation of the gastroduodenal tolerance to ketoprofen, flurbiprofen, racemic ketoprofen and paracetamol: A randomised, single-blind, placebo-controlled trial; J. Clin. Pharmacol., 38, 19S-24S (1998)/, which has been indicated in previously carried out animal experiments /DE 40 28 906 C2/.

Since the discovery of the nuclear transcription factor NF-κB before about one decade, extensive research works have been carried for the biological function and for the influencing of the NF-κB formation by endogenic and exogenic substances. Of the known pharmacological substances, hitherto inter alia glucocorticoids, such as

dexamethasone and prednisone, immune suppressives, such as cyclosporin, tacrolimus and deoxyspergualin in therapeutic concentrations have been described as effective on the NF-κB activation. For the metabolites intermediately formed in the case of the biochemical inversion of R-ibuprofen to S-ibuprofen, an inhibition of the NF-κB activation was also demonstrated for an R-ibuprofen coenzyme A thioester and speculatively assumed that also R-ibuprofen, via the known metabolic activation in the human body to the R-ibuprofen-CoA thioester, would show an action which R-ibuprofen itself does not possess. /Brune et al., Medicament containing ibuprofen thioester as inhibitor of the Nf-κB-dependent formation of mediators of inflammations and pain, DE 197 16 713 A1, WO 98/47502/7.

Surprisingly, it has now been found that other therapeutically used arylpropionic acid derivatives, such as flurbiprofen, ketoprofen, naproxan, tiaprofenic acid and fenoprofen, which display no noteworthy formation of CoA-thioesters in humans and, therefore, not racemising bring about an outstanding inhibition of the activation of NF-κB and thus possess the potential for the therapeutic effects associated with the influencing of this mechanism. In the following, this group is designated with "not racemising (abbreviated n.r.) R-arylpropionic acids".

The medicaments according to the invention based on n.r. R-arylpropionic acids and their derivatives as inhibitors of the NF-κB activation for the therapy of diseases which are influenced by the modification of the NF-κB activation are based on the following experimental investigations:

Fig. 2: Concentration-dependent influence of R- and S-flurbiprofen on the activation of the transcription factor NF-κB in RAW cells. The gel retention analysis (electromobility shift assay: DIG gel shift kit, Boehringer Mannheim) shows that LPS (1 µg/ml) leads to an activation of NF-κB (p50/p65 complex of NF-κB (trace No. 2 and 10). Macromolar concentrations of R-flurbiprofen (trace No. 3,4,5,6, 7 against trace No. 2 as control) were in the position to inhibit this LPS-induced activation of NF-κB. A densitometric evaluation showed that S-flurbiprofen was, with regard to these properties, about 100 times less potent (trace No. 11, 12, 13, 14 against trace No. 10 as control). Trace No. 1 and 8 each showed unstimulated control cells.

Since the nuclear transcription factor NF-κB is, inter alia, responsible for the formation of a series of enzymes with pro-inflammatory and oedema-forming properties, the influence of R-flurbiprofen on the zymosan-induced rat paw oedema was determined (Methods described by:

Meller S.T. and Gebhart G.F.: Intraplantar zymosan as a reliable, quantifiable model of thermal and mechanical hyperalgesia in the rat: European Journal of Pain, 1, 53-52 (1997). Figure 3a-c summarises the results.

Fig. 3a-c: Time-dependent increase of the rat paw volume (measured with a plethysmograph) after intraplanar administration of zymosan. After administration of zymosan /Meller and Gebhart (v. supra)/ into a rear paw of the rat, as indication of an inflammation, it comes to an increase of the paw volume (placebo group, administration of vehicle = phosphate buffer (PP)). On the basis of the inhibiting action of R-flurbiprofen on the NF-κB activation, in the case of dosages in the range between 1 and 27 mg/kg body weight (administration: intraperitoneal), a surprising decrease of the paw volume can be seen. This effect was especially marked between the 2nd and 6th hour after zymosan administration. Dexamethasone (0.5 mg/kg body weight), a known inhibitor of the NF-κB activation, was used as positive control. As expected, S-flurbiprofen also showed a reduction of the paw volume, whereby, however, this effect is explicable not via an inhibition of the NF-κB activation but rather via an inhibition of the synthesis of pro-inflammatory

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prostaglandins. S-Flurbiprofen is a known inhibitor of the cyclooxygenases.

Fig. 4: Summary of the effects of 9 mg/kg R-flurbiprofen, 9 mg/kg S-flurbiprofen and 0.5 mg/kg dexamethasone against placebo (V) over 24 hours. The effects after 9 mg/kg R-flurbiprofen were comparable with those after 0.5 mg/kg dexamethasone.

The preparation and chiral separation of the arylpropionic acids and of their derivatives is known. By way of example, reference is made to WO 93-17677 and the literature mentioned therein.

By arylpropionic acid derivatives, there are understood, according to the invention, the derivatives split back into arylpropionic acids in the stomach/intestinal tract (in the case of oral administration) or in the blood, such as alkyl esters with 1 - 6 C-atoms which can possibly contain amino or hydroxyl groups, amides or alkylamides with 1 - 6 C-atoms, as well as pharmaceutically compatible salts, especially alkali metal, alkaline earth metal, ammonium, amino acid salts, preferably lysinate, megluminate, trometamine, arginate or aluminium salts. Such compounds are also known.

The meaning of a prophylactic or therapeutic administration of n.r. R-arylpropionic acid in the acute or chronic treatment of diseases is varied corresponding to the severity of the ailment to be treated. The dose and

frequency of the dosings are also to be differentiated according to the age, body weight and reaction of the individual patients. In general, the daily dose of n.r. R-arylpropionic acid for the described ailments present lies between about 50 mg and about 2000 mg, administered in one or more doses. Preferably, the daily dose lies between about 100 mg and about 500 mg, administered in one or more doses. In the case of the care of the patient, the treatment should be begun with a lower dosing, possibly of 20 mg to 200 mg and increased up to about 1000 mg or higher, depending upon the general reaction of the patient. Furthermore, it is recommended that infants, children, patients over 65 years and those with impaired kidney and liver function first receive a lower dose and titrated based on the individual reaction and the blood level. In some cases, it can be necessary to use a dosing outside this range, which is obvious to the expert. Furthermore, it is to be noted that the treating house physician or clinical specialist knows, in conjunction with the general reaction of the patient, how and when the treatment is to be interrupted, adjusted or discontinued. The espression "an amount which is sufficient for the NF-κB inhibition but is not sufficient in order to initiate disadvantageous reactions (prostaglandin synthesis inhibition)" is included by the given dosage amounts and dosage instructions. Any desired form of administration can be used in order to provide

the patient with an effective dosing of the n.r. R-arylpropionic acid. For example, oral, rectal, transdermal, parenteral (subcutaneous, intramuscular, intravenous), intrathecal, epi- and peridural and similar forms of administration can be used. Possible forms of administration are e.g. tablets, dispersions, suspensions, solutions, plasters and the like.

The pharmaceutical formulations of the present invention include n.r. R-arylpropionic acid as active material or a pharmaceutically compatible derivative thereof and a pharmaceutically compatible carrier material and, if desired, other therapeutic derivatives.

The expression "pharmaceutically compatible derivatives" or "a pharmaceutically compatible derivative thereof" refer to derivatives prepared from pharmaceutically compatible, non-toxic acids or bases, including inorganic acids and bases and organic acids and bases. Since the component of the present invention is acidic, derivatives with pharmaceutically compatible, non-toxic bases, including inorganic and organic bases, can be prepared. Suitable pharmaceutically compatible basic additive derivatives for the components of the present invention include metal salts, prepared from aluminium, calcium, lithium, magnesium, potassium, sodium and zinc, or organic salts prepared from lysine, N,N'-dibenzylethylenediamine, choline, diethanolamine, ethylenediamine, meglumin (N-methylglucamine), tromethamine, arginine and alkylamines with 1 - 6 C-atoms.

The formulations of the present invention include formulations such as suspensions, solutions, elixirs and aerosols. Carrier materials, such as starch, sugar, microcrystalline cellulose, diluents, granulation adjuvants, lubricants, binding agents, solubilisers and the like can be used in the case of the solid oral forms of administration. Solid oral forms of administration (such as powders, capsules and tablets) are preferred to the liquid oral forms of administration. The preferred solid oral forms of administration are tablets. If desired, the tablets can be coated with standardised water or water-free coating agents.

In addition to the usual above-mentioned forms of administration, the component according to the invention can be administered with per se known agents in retarded inflowing and/or rapidly inflowing form. For example, hydrophobing additives to oral forms of administration act delayingly, disintegrating agents and tensides promote dissolving and thus acceleratingly and, as known, both forms can be mixed in granulate form in order to allow a part of the active material to flow in quickly and the rest delayed.

Pharmaceutical formulations of the present invention which are suitable for the oral form of administration can, as separate units, such as capsules, dragees or tablets, in each case contain a pregiven amount of the active material in the form of powder or granulate or as solution or suspension in an aqueous liquid, a non-aqueous liquid,

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an oil-in-water emulsion or a liquid water-in-oil emulsion. Such formulations can be prepared according to any pharmaceutical method but all methods comprise a mixing of the active material with a carrier substance which consists of one or more of the necessary components. In general, the formulations are prepared by uniform and thorough mixing of the active material with liquid carrier substances or finely divided solid carrier substances or both and then, if necessary, forming of the product into the desired form of administration.

For example, a tablet can be produced by pressing or forming, if desired with one or more additional components. Pressed tablets can be produced by pressing in an appropriate device when the active material is present in a friable form, such as powder or granulate, optionally mixed with a binding agent, lubricant, inert diluent, dispersing or surface-active agent. Formed tablets can also be produced by shaping of a mixture of the pulverised components, moistened with an inert liquid diluent, in a suitable device and subsequent drying. Preferably, each tablet contains between 50 mg and 1000 mg of the active material and each dragee or capsule contains between about 50 mg and about 600 mg of the active material. Especially preferably, the tablet, dragee or capsule contains one of four dosages, namely, 50 mg, 100 mg, 200 mg or 500 mg of the active material.